HYDROGEN PEROXIDE, PEROXIDASES AND LOW RANK COAL

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ABSTRACT

Horseradish peroxidase was tested to determine if it catalyzed any reaction between coal and hydrogen peroxide. Experiments were performed in aqueous buffers of either pH 5.0, 6.5 or 8.0 and used either particulate Wyodak, Beulah Zap, Texas lignite, Mississippi Wilcox lignite or solubilized Mississippi Wilcox lignite. Reactions were monitored by determining amounts of hydrogen peroxide consumed at various points in time. All coals reacted rapidly with hydrogen peroxide in the absence of peroxidase and these reaction rates increased as pH increased. Horseradish peroxidase did not measurably increase these reaction rates even when present in large concentrations. These data suggest that coal is not a substrate for horseradish peroxidase.

INTRODUCTION

Reports in the early 1980's that microorganisms were able to transform low rank coals into a liquid or water soluble form (1,2) generated interest in using bioprocessing as a possible route to convert coal into liquid fuels. Many organisms and enzyme systems have been examined since then for their ability to solubilize and/or depolymerize coal. Those enzyme systems that have received the most attention are those that catalyze the oxidative cleavage of carbon-carbon bonds in model compounds. These enzymes include laccases and peroxidases.

Laccase from Trametes versacolor (a.k.a. Coriolus versacolor and Polyporus versacolor) has been extensively studied (3,4). This enzyme utilizes molecular oxygen to cleave carbon-carbon bonds adjacent to aromatic nuclei (5) and has been implicated as the agent produced by T. versacolor responsible for the organism's coal solubilizing ability (1,3,4). Subsequent work indicated that the majority of coal solubilizing activity present in culture fluids was low in molecular weight (<1,000) and did not appear to be a laccase. A higher molecular weight material that had some coal solubilizing activity was found, but this was later identified as an esterase (6,7,8). It now appears that laccases are not significantly involved in coal biosolubilization.

Peroxidases are enzymes that utilize hydrogen peroxide and include horseradish peroxidase (HRP) and lignin peroxidases. When these enzymes are incubated with hydrogen peroxide (HP) and coal in either aqueous or nonaqueous solutions, coal biosolubilities increase (9,10). These increases have been attributed to the action of the peroxidases, but, since HP is quite reactive with coal, it is not known whether the rate of the enzyme catalyzed reaction is significant as compared to the nonenzymatic chemical reaction. In this manuscript we report on the relative rates of HRP catalyzed reactions as compared to the nonenzyme catalyzed chemical reaction.

EXPERIMENTAL

Mississippi Wilcox, Texas lignites were obtained as described and ground to -100 mesh (11). Beulah Zap (-100 mesh) and Wyodak (-100 mesh) were obtained from the Argonne Premium Coal Bank, Argonne, IL. Horseradish peroxidase type II (HRP) was obtained from Sigma Chemical Company, St. Louis, MO.

Solubilized Wilcox was obtained by placing 5g of coal in 1l of 50mM Tris buffer, pH 8.0. After mixing for several days, the suspension was centrifuged at 10,000xg for 20 minutes and the supernatant obtained. Solubilized coal was precipitated by acidifying the supernatant to pH 2 with HCl and allowing the suspension to stand for several hours. Precipitated coal was collected by centrifugation, washed several times with 1mM HCl and redissolved in 50mM Tris buffer. This solution was then filtered (Gelman type GA, 0.2µm pore dia.) to remove any insoluble material. The final concentration was approximately 2g coal/liter. Iron concentrations present in the solubilized coal was determined using an ARL Model 3520 atomic absorption spectrophotometer in the ICP mode.

Assays to determine relative rates of enzyme and nonenzyme catalyzed reactions were performed at 30°C and used either 1g of particulate coal or 5ml of solubilized coal. Reactions were initiated by the addition of 50ml of the appropriate buffer containing approximately 1mM HP. Actual concentrations of HP present were determined iodometrically (12). Buffers used were 500mM acetate, pH 5.0, 500mM phosphate, pH 6.5 and 500mM Tris, pH 8.0. At indicated time points, 50µl aliquots were removed from the reaction mixture and concentrations of HP present determined using the leucocrystal violet assay (13). Controls included HP with no additions, HP with 5mM resorcinol (positive control) and HRP, resorcinol with no other additions and HP with coal, resorcinol and HRP (positive control).

RESULTS AND DISCUSSION

Hydrogen peroxide, in the absence of HRP, reacted rapidly with all coals at each pH tested (Figure 1). Reactions profiles were similar in every case. Initial reactions were rapid and slowed as the reaction proceeded. This reaction appeared to be second order with respect to peroxide since a plot of 1/peroxide vs. time was linear (Figure 2). Assuming that this reaction was second order, values for the reaction constants could be obtained by determining slopes of lines generated by these plots (Table 1). Typically, these plots yielded lines with correlation coefficients 0.975 or better. As a general rule reactions were slowest at lower pH values and increased as pH increased. This is consistent with the literature indicating that hydrogen peroxide becomes a more powerful oxidizing agent as pH increases (14).

The reaction of HP with resorcinol as catalyzed by HRP was used as a positive control. This reaction not only demonstrated that HRP was active, but also gave an indication as to the activity of the enzyme with a preferred substrate and yielded some insight into the peroxidase reaction. Once again, a plot of 1/peroxide vs time yielded a straight line indicating that this reaction was second order with respect to HP (Figure 2). This result was obtained every time the experiment was performed and is consistent with earlier reports in the literature (15,16).

Because rates were determined by measuring HP disappearance, it was not known if the disappearance of HP was due to coal oxidation. Coals were not analyzed to determine if the coal itself was being oxidized because the amount of oxidation occurring would have been too small to measure using ultimate analyses. Other possible causes that could have accounted for the disappearance of HP were, therefore, investigated. An alternate material that might have been oxidized at the expense of HP would have been pyrite (17). The ability of HP to oxidize pyrite using the above conditions indicated that no significant rates of pyrite oxidation occurred at pH values of 5.0 and 6.5. At pH 8.0, significant amounts of HP were consumed in short periods of time indicating that pyrite was being oxidized (data not shown). Another possibility was that iron ions present in the coal could catalytically decompose HP (18). Solubilized Wilcox coal was analyzed and found to contain 1mM iron. Addition of 0.5mM and 1mM Fe^{+++} to solubilized Wilcox did not increase rates of HP consumption and the addition of 2mM Fe^{+++} resulted in only a slight increase in HP consumption (data not shown). These data suggest that the carbon present in the coal was being oxidized.

The addition of HRP to reaction mixtures containing both coal and HP generally had very little effect upon rates of HP consumption (e.g., Figure 1) and in most cases decreased rates of HP disappearance (Table 1). The lack of change in rates of HP consumption in the presence of coal was not due to the coal rendering the HRP inactive since HRP was able to oxidize resorcinol in the presence of coal. Also, the amount of enzyme in reaction mixtures containing coal was 10-fold greater than that present in positive controls containing resorcinol. Since no significant increases in rates of HP consumption were observed when large quantities of HRP were present in coal mixtures, then at least one of two possibilities must have existed. The first possibility was that HRP was unable to use coal as a substrate. A second possibility was that HRP was able to use coal as a substrate, but the number of sites where this could be done was extremely small which would have limited increases in HP consumption to below detection limits. Also, if there were such a small number of sites present, then increasing the amount of peroxidase present in reaction mixtures would not result in an increase in HP consumption.

Results from this study indicate that HRP is able to catalyze little or no reaction between coal and HP. Since peroxide reacts readily with coal and since HRP is an expensive enzyme, it would appear that continued use of HP in the presence of HRP would be of little value. This work does not preclude the possibility that other more powerful peroxidases (e.g., lignin peroxidases) might be able to catalyze the oxidative depolymerization of coal. A study involving the ability of lignin peroxidases to oxidatively depolymerize coal will be the subject of a subsequent manuscript.

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REFERENCES

1. Cohen, M. S. and P. D. Gabriele. 1982. Appl. Environ. Microbiol. 44:23.

2. Fakoussa, R. M. 1981. Ph.D. Thesis, Freidrich-Wilhelms University, Bonn, Federal Republic of Germany.

3. Cohen, M. S., W. C. Bowers, H. Aronson and E. T. Gray, Jr. 1987. Appl. Environ. Microbiol. 53:2840.

4. Pyne, J. W., D. L. Stewart, J. Fredrickson and B. W. Wilson. 1987. Appl. Environ. Microbiol. 53:2844.

5. Dodson, P. J., C. S. Evans, P. J. Harvey and J. M. Palmer. 1987. FEMS Microbiol. Lett. 42:17.

6. Campbell, J. A., D. L. Stewart, M. McCulloch, R. B. Lucke and R. M. Bean. 1988. Prep. Pap.-Am. Chem. Soc., Div. Fuel Chem. 33(4):515.

7. Pyne, J. W., D. L. Stewart, J. C. Linehan, R. M. Bean, M. A. Powell, R. B. Lucke, B. L. Thomas, J. A. Campbell and B. W. Wilson. 1987. Proceedings of the Biological Treatment of Coals Workshop, U.S. Department of Energy, pp. 174-194.

8. Bean, R. M., J. K. Fredrickson, J. A. Campbell, D. L. Stewart, J. A. Franz, B. L. Thomas, M. McCulloch, J. C. Linehan and B. W. Wilson. 1989. Proceedings: 1989 symposium on Biological Processing of Coal and Coal-Derived Substances, Electric Power Research Institute, pp. 3-1 - 3-25.

9. Scott, C. D. and S. N. Lewis. 1988. Appl Biochem. Biotech. 18:403.

10. Scott, C. D., C. A. Woodward, J. E. Thompson and S. L. Blankinship. 1990. Appl. Biochem. Biotech. In Press.

11. Quigley, D. R., C. R. Breckenridge, P. R. Dugan and B. Ward. 1989. Energy Fuel. 3:371.

12. Allen, A. O., C. J. Hochanadel, J. A. Gormley and T. W. Davis. 1952. J. Phys. Chem. 56:575.

13. Mottola, H. A., B. E. Simpson and G. Gorin. 1970. Anal. Chem. 42:410.

14. Sidgwick, N. V. 1950. Chemical Elements and Their Compounds, Vol II. Oxford University Press. pp. 868-874.

15. Chance, B. 1951. Advances in Enzymology. 12:153.

16. Chance, B. 1949. Arch. Biochem. 22:224.

17. Boron, D. J., A. G. Dietz and S. R. Taylor. 1981. Fuel. 60:991.

18. Walling, C. 1975. Acc. Chem. Res. 8:125.

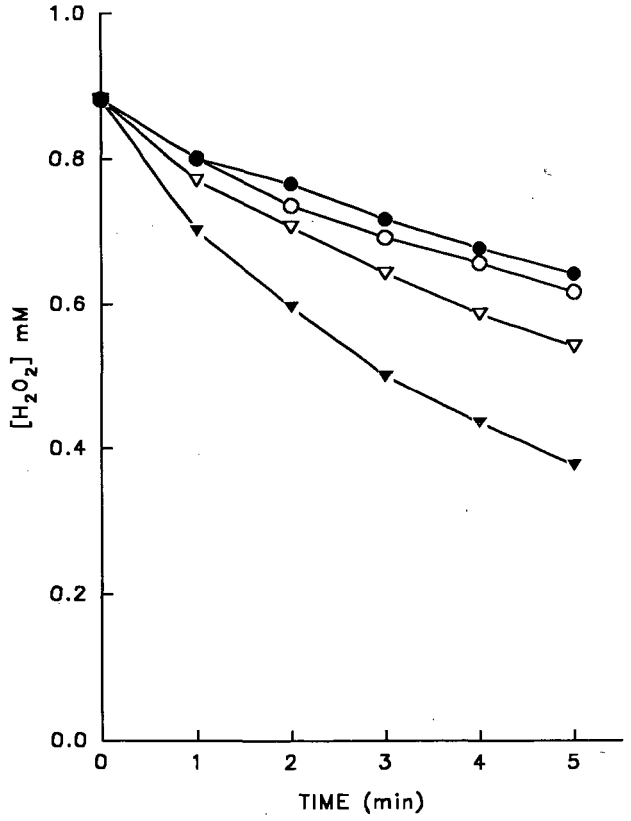

Figure 1. Disappearance of Hydrogen Peroxide in the Presence of Texas Lignite and Resorcinol at pH 8.0. Reaction mixtures are as described. Texas lignite = open circles; Texas lignite with HRP = closed circles; Resorcinol with HRP = open triangles; Texas lignite with Resorcinol and HRP = closed triangles.

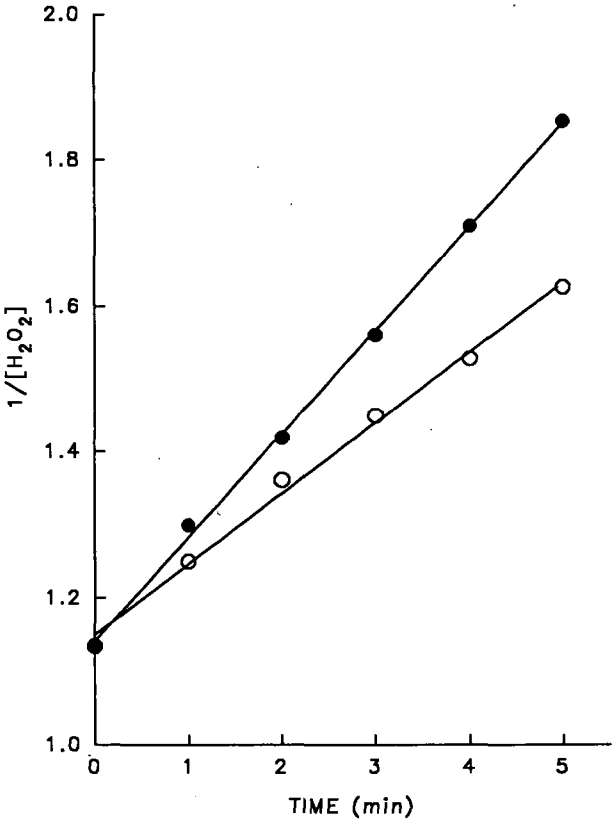

Figure 2. Plot of Inverse Hydrogen Peroxide Concentration vs. Time. Data taken from Figure 2. Correlation coefficients are 0.998 and 0.999 respectively for Texas lignite reacting with HP in the absence of HRP (open circles) and resorcinol reacting with HP in the presence of HRP (closed circles.

Coal		pH 5.0	6.5	8.0
Wilcox lignite	-HRP	0.078	0.146	0.269
	+HRP	0.053	0.112	0.319
Beulah Zap	-HRP	N.M.	0.029	0.064
	+HRP	N.M.	0.025	0.051
Texas lignite	-HRP	0.017	0.119	0.097
	+HRP	0.015	0.080	0.084
Wyodak	-HRP	0.073	0.058	0.109
	+HRP	0.045	0.039	0.099
Soluble Wilcox	-HRP	0.037	0.044	0.029
	+HRP	0.031	0.043	0.036

Table 1. Rate Constants for the Reaction of Various Coals and Hydrogen Peroxide in the Presence and Absence of Horseradish Peroxidase. -HRP indicates the absence of horseradish peroxidase; N.M. indicates not measured. Values are given as $(\text{mmoles/liter/min})^{-1}$.